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EFFECT OF SALT STRESS ON *ROSA* 'NEW DAWN' IN *IN VITRO* CULTURE

WPŁYW STRESU SOLNEGO NA KULTURY *IN VITRO* RÓŻY 'NEW DAWN'

Abstract: Salt stress is one of the main causes of plant damage in horticulture and tree die back in cities. It can be of natural origin but most often is due to human activity, eg uncontrolled fertilization or the use excessive amounts of salt for winter maintenance of streets and pavements. Plants are selected for resistance to salt, and laboratory *in vitro* techniques proved very useful for this purpose. The present experiments aimed to determine tolerance of roses *Rosa* 'New Dawn' to salt. The effect of sodium and calcium chloride on plant growth and shoot development in 'New Dawn' rose in *in vitro* cultures was examined. The plants were cultured on the QL medium containing 5 μM BA, 0.05 μM NAA, 0.3 μM GA_3 and 20 g · dm^{-3} sucrose, pH = 5.6, supplemented with NaCl or $CaCl_2$ (at concentrations 0–400 mM). The media containing increasing concentrations of salts under study showed inhibitory effect on regeneration and multiplication of rose shoots. $CaCl_2$ was less toxic than NaCl. An increase in medium salinity, independently of the salt tested, reduced the height of newly grown shoots and the number of leaves. Moreover, the proportion of chlorotic, deformed or necrotic leaves increased with the increasing salt concentration. The media did not affect shoot fresh mass index or fresh-to-dry mass ratio, which remained at the same level independently of chloride concentration in medium.

Keywords: *in vitro*, multiplication, NaCl, $CaCl_2$, salt stress, *Rosa* 'New Dawn'

Salt stress is an important factor causing plant damage in horticulture and tree die back in cities. High salt concentration in soil suppresses growth and development of plants and decreases productivity. About 25 % of arable land worldwide shows excessive salinity caused mostly by NaCl. It can be of natural origin but most frequently is due to human activity, eg uncontrolled fertilization or excessive use of salt for winter maintenance of streets and pavements. *In vitro* techniques can be used as a tool for studying of the mechanisms of tolerance of plants to different environmental stresses, including salt stress. The advantages of tissue cultures include quick regeneration of plants and easiness of control of ambient conditions [1–9].

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This study aimed to determine the effect of salinity, examined by medium supplementation with NaCl or $CaCl_2$, on growth and development of *Rosa* 'New Dawn' in *in vitro* cultures.

Materials and methods

Microshoots of *Rosa* 'New Dawn', 5 mm high, with 2–3 leaves were placed in 250 cm^3 Erlenmeyer flasks on a basic medium according to Quorin and Lepoivre (QL) [10] supplemented with growth regulators: 5 μM BA, 0.05 μM NAA, 0.3 μM GA_3, pH 5.6. The tested media contained NaCl or $CaCl_2$ at concentrations: 12.5, 25, 50, 100, 200 and 400 mM. Tested salt-free media were used as the control medium. In each combination of the medium 6 replication (each flash with 5 explants) were tested.

Cultures were maintained in a growth room at 25/23 ^oC (day/night) at 80 % humidity, with 16-hour day for 6 weeks. The following values were recorded after 6 weeks: percentage of regenerating explants, shoot multiplication index (number of newly developed shoots per 1 explant), mean shoot height[mm], mean number of leaves per 1 newly formed shoot. Quality of regenerated plants was inspected visually and deformations and growth anomalies were recorded. Leaves on each shoot were classified into three groups: necrotic (brown, dying out), chlorotic (characterized by chlorosis) and/or deformation of the leaf blade and of good quality – properly developed, vividly green in color. Share of each class was calculated. Shoot fresh mass was measured and used for calculation of fresh mass growth index G_v according to the formula: $G_v = m_k - m_p/m_p$, where: G_v – fresh mass growth index, m_p – initial mass, m_k – final mass. Dry mass of plants was also determined after drying at 60 ^oC to constant mass.

Data were calculated using a two-factor statistical method for independent variables. Statistical significance was evaluated using Duncan test with confidence level $\alpha = 0.05$.

Results and discussion

Soil salinity is an increasingly important burden in all urban areas. It is mainly caused by uncontrolled use of salt for winter street maintenance [8]. Sodium chloride is most often used for this purpose because of its common commercial availability, efficaciousness and cost-effectiveness. Calcium chloride can be an alternative because it is even more effective but has milder effect on plants, however, it is more expensive, as well [11]. The mechanisms of plant tolerance to salinity can be investigated with the use of *in vitro* techniques which are a good tool for studies of plant reaction to salt and for selection of salt-tolerant lines [5].

Roses belong to the medium salt-sensitive plants [12].

Increasing concentrations of the chlorides under study, namely sodium chloride and calcium chloride in the QL medium (0–400 mM) showed an inhibitory effect on regeneration and multiplication of rose shoots (Tables 1 and 2).

Table 1

The effect of salt type in the medium on *in vitro* shoot cultures of *Rosa* 'New Dawn'

Treatment	Share of regenerating explants [%]	Shoot multiplication rate
Control	100.0 c*	2.6 c
NaCl	89.6 a	1.5 a
$CaCl_2$	93.8 b	1.7 b

* Means designated with the same letters do not differ significantly at $\alpha = 0.05$.

Table 2

The effect of salt type and concentration on a share of regenerating explants of *Rosa* 'New Dawn' [%]

Salt concentration [mM] (Average of NaCl and $CaCl_2$)	0 Control	12.5	25	50	100	200	400
NaCl	100 c	100 c	100 c	100 c	100 c	93.8 c	43.8 a
$CaCl_2$	100 c	100 c	100 c	100 c	100 c	100 c	62.5 b

Calcium chloride proved to be less toxic than sodium chloride, namely, rose shoots demonstrated better regenerative and multiplicative capacities (62.5 % of regenerating explants) but the result did not differ significantly vs NaCl-treated explants (43.8 %) (Tables 1 and 2).

The presence of 50 and 100 mM calcium chloride in the medium stimulated development of rose shoots but the difference did not reach statistical significance (1.9–2.5 shoots per 1 regenerating explants). In contrast, medium supplementation with NaCl significantly lowered rose shoot multiplication index (Fig. 1).

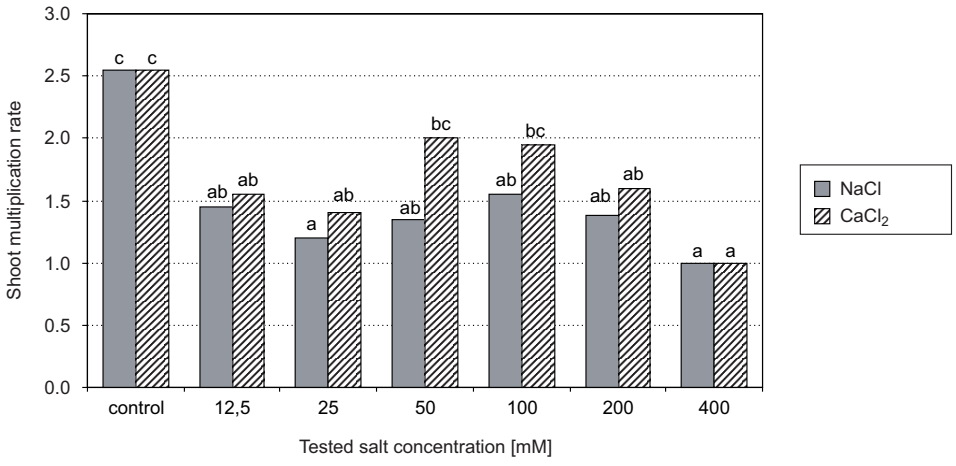

Fig. 1. The effect of salt type and concentration on multiplication rate of *Rosa* 'New Dawn' shoots

In *Populus euphratica in vitro* cultures, high NaCl concentrations (100–250 mM) inhibited callus growth but no die back was observed, however, the callus obtained on the high-salt medium differentiated very weakly and axillary buds did not develop [6]. Kuciakowski [13] in his studies on *Citrus limon* observed growth inhibition and die back of nucellar embryos on the medium containing 86 mM NaCl.

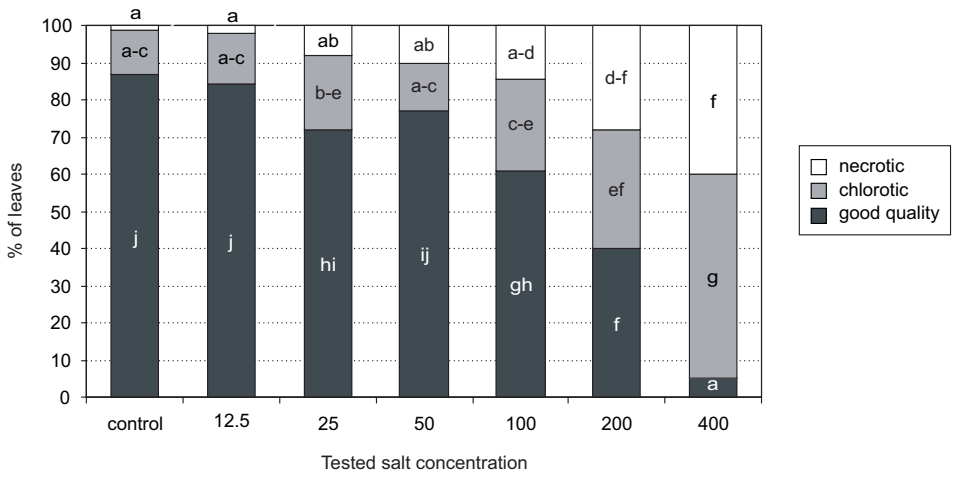

Fig. 2. The effect of average of tested salt concentration (NaCl and $CaCl_2$) on quality of leaves in *Rosa* 'New Dawn' regenerates

Zhang et al [6] observed maximal growth rate of *Populus euphratica* callus at 50 mM NaCl concentration. The difference in the reaction to salt between *Populus euphratica* plant and callus has not been explained. Vitagliano et al [14] investigated the effect of salt on *Cydonia oblonga* rootstock regeneration using shoot cultures, callus and cell suspension. The studies showed greater sensitivity of cell cultures, containing single cell suspensions, to salt than shoot and callus cultures.

Our studies on the effect of medium supplementation with NaCl or $CaCl_2$ on development of roses in *in vitro* cultures demonstrated a significant influence of salt concentration on mean length of newly formed shoots, independently of salt composition (Table 3). Shoots developed on the control tested salt-free medium were the longest (7.3 mm) in comparison with the growing on different concentration of NaCl (4.9–2.8).

Table 3

The effect of salt concentration in the medium on plant height and number of leaves in *in vitro* cultures of *Rosa* 'New Dawn'

Salt concentration [mM] (Average of NaCl and $CaCl_2$)	0 Control	12.5	25	50	100	200	400
Mean shoot height [mm]	7.3 c	4.9 b	4.4 b	4.9 b	3.2 a	2.5 a	2.8 a
Mean number of leaves/shoot	8.1 cd	8.9 d	8.4 d	7.2 c	5.9 b	5.2 b	4.1 a

Statistical analysis indicated that independently of salt type, its concentration affected the mean number of leaves per 1 regenerating shoot. The greatest number of leaves per regenerating shoot was observed on QL media containing 12.5 and 25 mM $CaCl_2$ (7.3, 8.4), and 12.5, 25 and 50 mM NaCl (8.5; 7.0; 7.7 leaves, respectively). The results obtained at these concentrations did not significantly differ from control (8 leaves) (Table 3).

The present experiment indicated worsening of quality of rose shoots with increasing chloride concentration in the medium. Visual analysis of rose leaves demonstrated a decrease in the fraction of good-quality, healthy, deep-green leaves with increasing salt concentration in the medium while the fraction of chlorotic, deformed and necrotic leaves rose was increased. The plants cultured on the control medium and medium containing 12.5 mM salt had the greatest number of healthy leaves (87–85 %). High NaCl concentration in the medium has been also shown to increase necrosis of poplar shoots [15].

Likewise, according to Wahone et al [4], an increasing salt concentration (5–30 mM) also elevated the number of damaged leaves in two varieties of *Rosa hybrida* 'Kiss' and 'Cardinal'. Those studies demonstrated high salt tolerance in the varieties of *Rosa hybrida*. The variety 'Kiss', was more salt resistant than 'Cardinal', since less than 50 % of its leaves were damaged on the medium supplemented with 0–10 mM salt while the variety 'Cardinal' had more than 50 % of necrotic leaves when cultured on the medium containing 5 mM NaCl. Increasing NaCl concentration in the medium can also lead to the accumulation of Na^+ and Cl^- ions, as observed in the varieties of *Rosa hybrida*: 'Cardinal' and 'Kiss' and in *Rosa chinensis* 'Major' and *Rosa rubiginosa*. The increased leaf damage can be attributed to the elevated chlorine concentration in these organs [4]. The damage of tissues, shoots and leaves in *Citrus limon* and tomato can be also caused by the excessive accumulation of Na^+ and Cl^- ions [13, 16].

Shoot growth index (Gv) did not significantly differ between plants cultured on media containing increasing salt concentrations and was similar as in control (Table 4).

Table 4

The effect of salt concentration in the medium on Gv and dry mass of regenerated plants of *Rosa* 'New Dawn'

Salt concentration [mM] (Average of NaCl and $CaCl_2$)	0 Control	12.5	25	50	100	200	400
Gv	3.3a	3.4a	3.0a	3.1a	2.9a	—	—
Dry mass [%]	12.9a	10.7b	10.0b	13.6a	12.6a	—	—

Wrochna et al [17] demonstrated that the presence of salt in medium stimulated fresh mass accumulation in *Amaranthus paniculatus*, *A. caudatus, Atriplex hortensis* and *Tamarix tetrandra.* This is a typical reaction of facultative halophytes, which need a low salt content for optimal growth [18].

The present experiments showed the effect of sodium and calcium chloride in medium on dry mass content. Tissues of roses cultured on the control medium contained 12.0 % of dry mass and did not significantly differ from tissues which were

cultured on the medium supplemented in 50 or 100 mM salt (12.6–13.6 %). However, dry mass content in plants cultured on these media was lower. Likewise, in *Cydonia oblonga* callus, the reduction of both fresh and dry mass was observed when cultured on the media supplemented with increasing NaCl concentrations, but fresh-to-dry mass ratio remained constant [14].

Conclusions

1. Increasing concentrations of sodium and calcium chloride (0–400 mM) in QL medium inhibited regeneration and multiplication of rose shoots, but $CaCl_2$ was less toxic than NaCl.

2. The salts under study reduced the mean length of newly developed shoots; the shoots were the longest on the control chloride-free medium.

3. Fraction of good-quality, healthy, deep-green leaves decreased and fraction of chlorotic, deformed and necrotic leaves rose enhanced with increasing both tested salt concentrations in the medium.

4. The present experiments demonstrated that an *in vitro* technique can be efficiently used for studies of the reaction of *Rosa* 'New Dawn' to sodium and calcium chloride.

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WPŁYW STRESU SOLNEGO NA KULTURY *IN VITRO* RÓŻY 'NEW DAWN'

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Abstrakt: Stres solny jest jednym z głównych czynników powodujących uszkodzenia roślin w uprawach ogrodniczych, a także obumieranie drzew w miastach. Może być wynikiem naturalnych procesów, ale najczęściej zachodzi pod wpływem działalności człowieka, np. nieracjonalnego nawożenia lub używania zimą dużych ilości soli w celu usuwania oblodzenia dróg i chodników. Przydatna w badaniu selekcyjnym reakcji roślin na zasolenie okazała się technika laboratoryjna *in vitro*. W przeprowadzonych doświadczeniach określano tolerancję róż *Rosa* 'New Dawn' na zasolenie. Oceniono wpływ chlorków: sodu i wapnia, na wzrost i rozwój pędów bocznych odmiany 'New Dawn' w kulturach *in vitro*. Rośliny były uprawiane na pożywce wg QL, zawierającej 5 µM BA, 0,05 µM NAA, 0,3 µM GA_3 oraz 20 g · dm^{-3} sacharozy, o pH = 5,6, wzbogaconej dodatkowo w NaCl lub $CaCl_2$ (w stężeniach 0–400 mM).

Wykazano hamujący wpływ pożywek ze wzrastającym stężeniem badanych soli na regenerację i namnażanie pędów róży, przy czym $CaCl_2$ działał mniej toksycznie w porównaniu z NaCl. Wzrost zasolenia pożywki, niezależnie od testowanej soli ograniczał wysokość nowopowstałych pędów i liczbę formujących się liści. Ponadto ze wzrostem stężenia soli zwiększała się liczba liści z chlorozą, a także zdeformowanych i nekrotycznych. Nie zaobserwowano wpływu badanych pożywek na współczynnik wzrostu świeżej masy pędów róży, podobnie stosunek świeżej masy do suchej masy pozostawał na tym samym poziomie, niezależnie od stężenia chlorków w pożywce.

Słowa kluczowe: *in vitro*, namnażanie, NaCl, $CaCl_2$, stres solny, *Rosa* 'New Dawn'